

NOTES AND BRIEF ARTICLES

CLOSURE FOR CULTURE BOTTLE

Aerobic micro-organisms that grow rapidly in surface culture often need a larger opening in the culture vessel, in proportion to the growth surface area, than micro-organisms that have a slow rate of growth. It is, therefore, frequently necessary to grow micro-organisms in wide-mouth bottles rather than narrow-mouth bottles to obtain greater interchange of air. A cotton plug could be used as a closure for the wide-mouth bottle, but it has well-known disadvantages. We have devised the bottle cap assembly shown in FIG. 1 as a substitute for the cotton plug. This assembly is made from a standard molded plastic cap, filter paper, a rubber band, and a prepared glass sleeve. The top of the plastic cap is cut out, so that the diameter of the opening is approximately equal to the diameter of the bottle mouth. Sufficient edge is left on the cap to give a good seal with the glass edge of the bottle. A snug fitting flat rubber band is placed on the outside of the cap to prevent the glass sleeve from slipping, and also act as a cushion to close crevices in the folded edge of the filter paper. The cap is screwed on the bottle and covered with filter paper and the glass sleeve is then placed over it.

For our assembly, Schleicher and Schuell¹ sharkskin filter paper was used. It has high wet strength, resistance to repeated steam sterilization and good air permeability. The number of filter papers used should be sufficient to give a tight fit between the glass sleeve and the cap; a minimum of three pieces is recommended. As many as twelve pieces can be used without significant effect on the growth of micro-organisms. It may be necessary to add more filter paper to some assemblies after repeated use to maintain a tight fit between the glass sleeve and the cap.

The glass sleeve is made long enough to reach the shoulder of

¹ Mention of brand names does not constitute endorsement or recommendation by the Department of Agriculture over similar brands not mentioned.

the bottle. This reduces the risk of entrance of contaminating particles, and also permits the assembly to rest on the table top while the cap is kept a safe distance above the table top. The top edge of the glass sleeve is turned in to decrease deposition of dust and foreign particles on the filter paper.

With this bottle closure, it is possible to inoculate one or a group of bottles very simply. The closure is unscrewed beforehand, but left on the top of the bottle. After addition of the inoculum, the

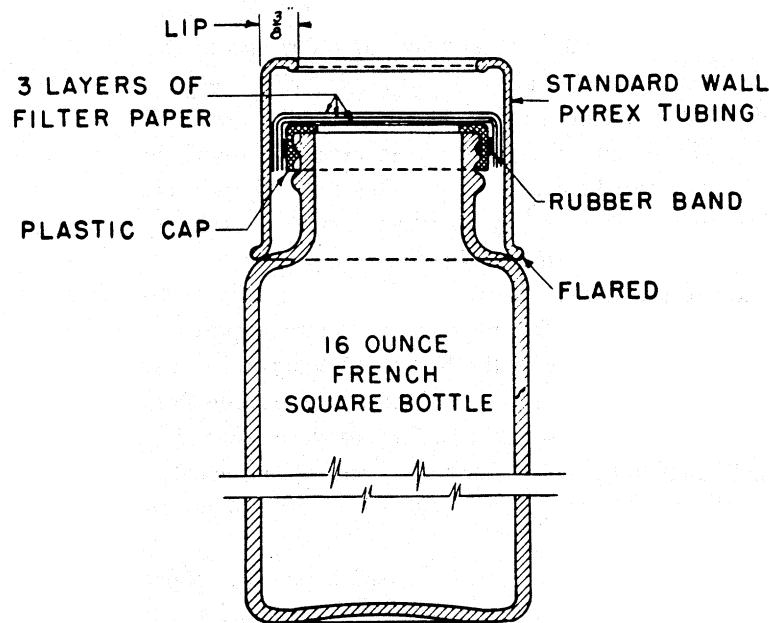


FIG. 1. Bottle with bottle cap assembly.

closure can again be placed on the bottle top, and screwed down later.

Use of this bottle closure has made it possible to obtain excellent spore crops from strains of *Aspergillus niger* and *Aspergillus oryzae* in a short time. Culture of these organisms on media supporting excellent growth in containers not having wide mouths closed with material of good air permeability showed suppressed spore formation.

Media cannot be stored for any length of time in wide-mouth bottles capped either with cotton plugs or the closure described herein without loss of water. We therefore explored the possibility of using the assembly with only a hole drilled in the top of the cap instead of having the entire top removed. This would restrict the loss of water and perhaps have little effect on the spore production of the micro-organism. A clearance between the top of the cap and the filter paper of at least $\frac{1}{16}$ of an inch was effected by a rubber washer $\frac{1}{8}$ inch wide and equal in diameter to the diameter of the cap. Sixteen-ounce French square bottles containing 50 mls. of whole potato media (7% solids) were inoculated with spores of *Aspergillus oryzae* NRRL 458 and incubated for 4 days at 30°C. Spore counts per bottle are shown below.

	Spores per bottle, billions
Closure having 1.5-inch hole (top of cap removed)	9.00
Closure having $\frac{1}{16}$ -inch hole in cap	0.85
Closure having $\frac{1}{8}$ -inch hole in cap	1.40
Closure having $\frac{1}{4}$ -inch hole in cap	2.30
Closure having $\frac{1}{2}$ -inch hole in cap	3.00

The spores in the bottles having holes less than 1.5 inches in diameter in the caps were smaller and lacked characteristic color.

The bottle closure described can be constructed easily and can be used over and over again without loss of its effectiveness. For quantity production, a solid plastic piece could be used in place of the glass sleeve and the plastic cap. The filter paper could then be cut to fit the inside of the cap opening, and held against it by a plastic ring or other suitable means. This arrangement would make it convenient to replace the filter paper. A closure of this type should be useful for many containers employed in culture work.—ELMER A. WEAVER, THEONE C. CORDON AND HARRY J. JOHN, Eastern Regional Research Laboratory, Philadelphia 18, Pennsylvania.